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A Study on the Effects of Cellulase, Xylanase and Molasses Enzymes on the Nutritional Value of Common Reed Silage Using the Gas Production Test and Dacron Bags Methods

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Abstract- This research aims to determine the nutritional value of common reed silage using the nylon bags and gas production test methods. Treatments under study include: 1- common reed silage with no additives (control group), 2- common reed silage + 2.5 g fiber degrading enzymes (cellulase and xylanase), 3- common reed silage + 15% molasses, and 4- common reed silage + 2.5 g enzyme mixture and 15% molasses. Use of the standard method for chemical components, the nylon bags method for dry matter degradation, and the gas production method for organic matter digestibility and metabolizable energy were determined. The results indicated that CP amounts of treated groups showed no significant difference with control group ($P>0.05$). The experimental group containing 15% molasses and 2.5 g enzyme mixture showed the least amounts of ADF and NDF while the control group showed the most amounts of ADF and NDF ($P<0.05$). Also, the experimental groups showed significant differences at different incubation intervals regarding the degradation ($P<0.05$). The experimental group containing 2.5 g enzyme mixture + 15% molasses had the most degradation amount while the control group had the least. Results from the produced amounts of gas (mg per ml) at different incubation intervals of the studied treatments indicated that the treatment containing 2.5 g enzyme mixture + 15% molasses produced the most amounts of gas and was most digestible while the control group produced the least amounts of gas and was least digestible. Considering the parameters of degradation and digestibility in this research, it can be concluded that common reed silage processed with 2.5 g enzyme mixture + 15% molasses produces the best results.

Keywords- *Common reed, Nutritional value, Fiber degrading enzymes, Molasses*

I. INTRODUCTION

Hamoon Wetland canebrakes are a very rich food source for animal nutrition. These canebrakes stretch over 12287 hectares (Puzak, Saberi, and Hirmand) and the dominant species there include Reed, Typhaceae, Arundo, Butomus and sedge (Asadi moqadam and Nik khah, 1992). From among 28 species, reed has the frequency of 28% which makes it the first plant in that regard and local farmers gather it in mid-July when the plant matures and store it after it dries. Then in autumn and winter, this dried forage will be used as animal food (Nuri et al, 2007). In recent years, the use of molasses and fiber degrading enzymes as a useful additive to the ruminant animals' food which consumes a lot of fiber has gone through extensive studies and researches (Murgavi, 2000). Cellulase and xylanase enzymes have been given much more consideration in this regard because most of the indigestible parts of the cell walls are made of lignin and cellulose and these enzymes can degrade lingo cellulosic bonds and therefore making them more exposed to ruminal microorganisms which results in better digestion (Buchmin et al, 1995; Tang, 2008). The most difficult problem to deal with is the low digestibility of reed and its high amount of crud fibers. Aqa shahi (1995) reported that molasses increases the digestibility of reed silage. Thus, with a new and scientific idea (simultaneous use of molasses and fiber degrading enzymes as additives in reed silos) the present study seems essential. The aim of this research was to add molasses and enzyme mixtures to reed siloes and study its effect on the nutritional value, degradation parameters, and gas production.

II. MATERIALS AND METHODOLOGY

A. Sampling

In order to study the nutritional value, degradation parameters, and gas production of common reed silage, the nylon bags and gas production techniques were used. Treatments under study include: 1- common reed silage with no additives (control group), 2- common reed silage + 2.5 g enzyme mixture (cellulase and xylanase in the proportion 50:50 (enzyme mixture)), 3- common reed silage + 150 g (15% molasses), and 4- common reed silage + 2.5 g enzyme mixture and 15% molasses per each kg of the primary dried matter. In order to prepare the silage, the samples were collected from the forages around Hamoon Lake in May 2009 while the plants were at seed dissemination stage. After sampling, the samples were cut into 3-5 cm pieces and for each treatment with 3 repetitions, they were stored in 5 liter buckets for silage. The

buckets were opened after 27 days. The pH was measured at the exact time of opening using fitful (2002) method. To measure the chemical component, degradation parameters, and gas production of the samples, they were kept in the Avon at 60˚c for 48 hours to dry off and milled using a miller equipped with a sieve of 2 mm diameter. The chemical component of experimental groups [including organic matter (OM), crud protein (CM), ether essence (EE), and ash] was done using the proposed standard method AOAC (1990). The insoluble fiber in NDF and ADF was measured using the proposed method by Van Suset et al (1991). And Bois et al phenol-sulfuric method (1993) was used to measure the water soluble carbohydrates (WSC).

B. Degradability of Nutrients

Degradability of the dry matter was determined using three local bulls which had fistula incubated in their rumen. The bulls' ration was determined at the maintenance level calculated according to NRC 2000 tables using regular food (dry alfalfa, hay, barley, bran, and cotton seed meal) and the adaptation period was two weeks. The bulls were fed two times a day at 6 and 18 o'clock and water was always available for them. An amount of 5 grams of milled and dry sample was put in Dacron bags made of poly ester fibers by the size 15*8 cm having 50 μm pores (Orskov et al, 1980). The bags were put in the bulls' rumen for 3, 6, 12, 24, 48, 72, and 96 h and after that each indicated hour passed, they were taken out, washed, and dried in the Avon at 65˚c for 48 hours. The degraded amounts were measured by calculating the difference between the primary sample and the residue in the bag. Degradability parameters (solution part, insoluble part, and degradation rate constant) was studied according to the exponential equation [P=a+b(1-r-ct)] (Orskov et al, 1980). In this equation, *P* is the degradability percentage, *t* is the interval, *a* is the intercept at the time 0 and the indicator of soluble matters, *b* is the slow fraction of degradation, *(a+b)* is the fraction that is potentially degradable, and *c* is the degradation rate (percentage per hour). The effective degradability of rumen (ED) was calculated according to ED=a+[b×c/c+k]. In this formula, *k* is the speed by which the food passes through rumen; here, it is decided to be 0.02.

C. Gas Production Test

Determination of laboratory fermentation and the amount of gas production was done using the method by Menke and Steingass (1988). To do this, rumen emulsion of local bulls (fistulated and castrated) was obtained. And an amount of 210 ±5mg sample (3 repetitions) was poured into every syringe and 30 ml of buffered and limpid rumen liquid was added to it and they were put in the incubator at 39˚c. The amount of gas production at hours 2, 4, 8, 12, 16, 24, 48, 72, and 96 was measured and recorded. The data on cumulative gas production was analyzed using this equation: Y=b(1-e-ct) in which *b* is the gas production from insoluble and non-fermenting fraction (ml in 200 mg dry matter), *c* is the constant gas production ratio for fraction b (h-1), *t* is the incubation time, and *Y* is the produced gas in t (Getchu et al, 2004). To estimate the organic matter digestibility (OMD) and the metabolizable energy (ME), the Menke and Steingass (1988) pattern was used as follows:

OMD = 14/88+ 0/8893 GP +0/448 CP+ 0/0651XA

ME= 2/2 +0/1357 GP +0/057 CP + 002859 CP2

OMD is the organic matter digestibility (percentage), *GP* is the gas production volume corrected for 24 h (ml in 200 mg dry matter), *CP* is the crud protein (dry matter percentage), *XA* is the crud ash (dry matter percentage), *ME* is the metabolizable energy (MJ in dry matter kg).

D. Calculations and Statistical Analysis

The acquired data for chemical components, degradability of dry matter, and gas test parameters went through statistical analysis in a random pattern. The data were first tested with Minitab version to make sure they are normal and then, using the SAS (2002) statistical software, they were statistically analyzed using the GLM method. Next, the averages were compared using the Duncan's multiple range tests assuming an error of less than 0.05. The results of in situ test and gas production were analyzed using the software Neway and Fitcurve respectively.

III. Results and Discussion

A. Chemical Components

Table 1 shows the chemical components of the experimental treatments of common reed silage. The amount of dry matter for the samples used in different experimental groups was significantly different in the range of 27.75 and 34.20 ($p<0.05$) in which the treatment with most amount of dry matter was the one with 2.5 g enzyme mixture and 15% molasses while the least amount of dry matter was in the silo with no additives. The results of this study showed that the addition of molasses and enzyme mixture increases the amount common reed silage dry matter ($P<0.05$). Alikhani et al (2005) and Kamali (2004) reported that treatment of the sunflower silage and tomato silage with molasses significantly increased the amount of dry matter in comparison with the control group.

The amount of common reed dry matter for different treatments differentiates in a range of 86.66 and 88.63 %. The least amount belongs to the control group and the most is that of the experimental group with 15% molasses and 2.5 g enzyme mixture ($p<0.05$). This finding is in agreement with findings of Alikhani et al (2005) on the treatment of sunflower silage. The ash average of treatments showed a reverse relationship with averages of organic matter in a way that the most amount of dry matter was that of the control group. Mashayekhi and Qorbani (2005) used different amounts of molasses in reed siloes and found similar results.

Addition of both molasses and enzyme mixture to the reed siloes did not result in any significant difference in the amount of proteins ($p>0.05$). The most amount of crud protein was that of the treatment with 2.5 g enzyme mixture and 15% molasses (8.13) while the least amount belonged to the control group (8.07). Several researchers have reported the increase of crud protein with the addition of molasses to the sorghum silo (Mehtap et al, 2007). Kung et al (2000), in their research, concluded that the effect of fibrolytic enzyme on the forage crude protein is insignificant which is in agreement with findings of the present study. The amount of crud fat was in the

range 2.21 and 2.94 % in different treatments and the most amounts belongs to the treatment with 2.5 g enzyme mixture and 15% molasses while the least belongs to the control group. Some researchers have reported that although the silo process may decrease the cell wall ingredients, it may result in the increase of crud fat (Hassan et al, 2005). Chilliard et al (2001), in their study, reported that ensiling the grasses results in the release of fatty acids by the triglycerides and no other change happens to them except in the case of having unwanted fermentation. The most amount of cell wall (NDF) was that of the control group (74.74%) while the least amount was that of the treatment with 2.5 g enzyme mixture and 15% molasses (56.20%). Ensiling results in a significant decrease of this variable ($p<0.05$) which is in agreement with findings of Hassan (2005) on banana waste silage. In silages with molasses and enzyme mixture, it results in greater decrease of the cell wall. Rezaee et al (1388) reported that by increasing molasses percentage in tumbleweed forage siloes, cell wall (NDF) decreased. Researchers have also reported that by adding the fiber degrading enzymes, cell wall has decreased (Morgavi et al, 2000). The in digestible fraction of cell walls is made of lignocellulose. Cellulase and xylanase enzymes can break these lignocellulose bonds and therefore better exposing them to ruminal microorganisms for digestion (Tang et al, 2008; Colombto, 2000). Furthermore, there seems to exist a kind of synergic relationship between molasses and the enzyme which if used together, makes them even more effective and results in an increased cell wall reduction amount of silo samples. The lowest percentage of cell wall with no hemicellulose (ADF) was that of the treatment with 2.5 g enzyme mixture and 15% molasses (34.29%) while the highest percentage of cell all with no hemicellulose was that of the control group (46.05%) ($p<0.05$). Xing et al (2008) have reported, in a study, the decrease of both ADF and NDF by adding fiber degrading enzymes. The average amount of the percentage of water-soluble carbohydrates showed a significant difference among different treatments ($p<0.05$). The highest percentage of water-soluble carbohydrates was that of the treatment with 2.5 g enzyme mixture and 15% molasses (14.4%), while the least amount was that of the control group (9.13%). The results of this study clearly indicated that the addition of molasses and enzymes results in a significant increase of water-soluble carbohydrates of the common reed silage. While degrading a fraction of NDF and ADF, the cellulase and hemicellulase enzymes release some amounts of water-soluble carbohydrates (Kulumbto et al, 2003). Addition of molasses and enzymes did not affect the amounts of calcium and phosphorus of the experiment groups which could be justified by considering the chemical components of molasses and fiber degrading enzymes. As it can be seen in table 1, adding molasses and enzymes (as the sugar source) to the silage results in a significant decrease of pH ($P<0.05$). All of the treatments, except for the control group, had suitable color and pleasant smell. Furthermore, no mold contamination was seen. The control group, however, had unpleasant smell and bad color (dark brown). Kuhshahi et al (2006) reported that, in a study on eggplant-shrubs silos, adding molasses would make the silage more stable and reduction of pH is because of an increase in the amounts of fermentable carbohydrates in the silage which is in agreement with the findings of Demirel et al (2003) in their study on sorghum silage.

TABLE I. CHEMICAL COMPONENTS OF REED-FORAGE IN DIFFERENT TREATMENTS

Average of the chemical component percentage											
Treatment	DM	Ash	OM	CP	EE	NDF	ADF	WSC	pH	ca	p
Control group	27.75^{c}	13.34^{a}	86.66^{c}	8.07^{b}	2.21^{b}	74.74^{a}	46.05^{a}	9.13^{c}	5.9^{a}	0.317	0.030
With 2.5 g enzyme	30.04^{b}	12.87^{b}	87.13^{b}	8.11^{a}	2.86^{a}	57.95^{b}	39.99^{b}	12.57^{b}	4.85^{b}	0.313	0.030
With 15% molasses	30.04^{b}	12.87^{b}	88.54^{a}	8.11^{a}	2.86^{a}	56.20^{c}	38.42^{b}	12.57^{b}	4.85^{b}	0.030	0.030
With 2.5 g enzyme + 15% molasses	34.20^{a}	11.37^{c}	88.63^{a}	8.13^{a}	2.94^{a}	56.20^{c}	34.29^{c}	14.40^{a}	4.71^{c}	0323	0.031
SEM	1.14	0.031	0.97	0.03	0.079	4.25	2.02	0.24	0.092	0.011	0.001

*The averages with similar numbers in each column have no significant difference ($P<0.05$).

*DM: dry matter, Ash: crud ash, OM: organic matter, CP: crud protein, EE: ether essence, NDF: neutral detergent fiber, ADF: acid detergent fiber, WSC: water-soluble carbohydrates.

B. Dry Matter Degradability

Table 2 shows the parameters for dry matter degradability of the experimental groups. The results of this study indicated that all treatments increased the parameter a of reed silage ($P<0.05$). The highest amount of parameter a belongs to the treatment with 15% molasses and the least amount is that of the control group (42.93 and 14.84 percent respectively) which is related to the water-soluble and easily digestible carbohydrates of molasses. This finding is in agreement with Khorasani (2010) who reported that adding molasses to date-branches' silos (at the level of 5%) increases the parameter a of the silage. The highest amount of parameter a was that of the control group (40.54) and the least amount was that of the treatment with 2.5 g enzyme mixture (23.33). this may be a result of having high amounts for parameter a because there will be less fiber in processed silages in comparison to unprocessed ones but a fraction of the fiber which remains may be less digestible.

(Kung et al, 2002) have reported that the cellulase enzyme first attacks the easily digestible fractions of the fiber which leaves the residue with fractions that are less digestible. The highest amount of parameter c is that of the treatment with 2.5 g enzyme mixture + 15% molasses (0.029) and the least amount is that of the control group (0.012). This treatment showed a significant difference with others which could be a result of the lower parameter a of reed silage. It is because of the reason that deficiency of parameter a causes the ruminal microorganisms to grow slower which in turn slows down the whole food degradation in the rumen (Danesh mesgaran et al, 2008). All of the treatments increased the effective degradation (ED) factor of reed silage ($P<0.05$). The highest amount of ED was that of the treatment with 2.5 g enzyme mixture + 15% molasses (55.03) and the least amount was that of the control group (30.46). Generally speaking, although doubtlessly the fiber degrading enzymes do increase soluble carbohydrate amounts of the forage, they affect digestibility on a lesser scale. Autrey et al (1975) added the trichoderma cellulase (0, 0.5, 1, 2 g per kg corn-forage). Although, in this study, the digestibility of processed forage with higher amounts of enzyme was seen in the cows, the differences were not significant. Still, most of the researchers have reported on the positive effects of using molasses and the improvement of digestibility percentage. Arbabi et al (2008) have reported that by increasing the molasses percentage in foxtale millet silos, the effective degradability increases too which is probably the result of high degradability of easily digestible molasses carbohydrates and it is in agreement with the findings of this research.

TABLE II. DRY MATTER DEGRADABILITY OF REED FORAGE SILAGE PROCESSED WITH ENZYME MIXTURE AND MOLASSES

Incubation Time (h)	Control group	Treatment with 2.5 g enzyme	Treatment with 15% molasses	Treatment with 2.5 g enzyme + 15% molasses	SEM
a(dry matter %)	14.84^{d}	32.03^{c}	42.93^{a}	39.23^{b}	0.76
b(dry matter %)	4.54^{a}	23.33^{b}	23.64^{b}	27.04^{b}	1.90
a + b	55.38^{c}	55.36^{c}	66.58^{a}	66.26^{a}	1.85
c (% per hour)	0.012^{d}	0.016^{c}	0.022^{b}	0.029^{a}	0.004
ED (% per hour)					
0.02	30.46^{c}	49.73^{b}	55.40^{a}	55.03^{a}	0.41
0.05	23.23^{d}	44.66^{c}	50.26^{a}	49.06^{b}	0.19
0.08	20.36^{d}	42.30^{c}	48.10^{a}	46.36^{b}	0.22

*In this table, a: soluble particles (quickly degradable fraction), b: insoluble particles (slowly degradable fraction) which are potentially degradable, C: degradation rate constant (percent per hour), a + b: the overall percentage of food that gets degraded in the rumen (in other words, a + b is the potential degradability of food in the rumen), ED: shows the effective degradability of food in the rumen (percent per dry matter).

*Numbers with similar characters in each row have no significant difference in statistical terms ($P<0.05$).

*SEM: shows the standard error of measurement for the averages.

C. Gas Production Test

Table 3 shows the average amount of produced gas (ml in 200 mg dry matter) at different hours of incubation (2, 4, 6, 8, 12, 24, 48, 72, and 96). As the incubation time increases, the amount of produced gas increases too which is in agreement with other studies (Mansuri et al, 2003). The average amount of produced gas at the hour 96 of incubation varies from 47.28 to 50.83 ml among different treatment in which the treatment with 2.5 g enzyme mixture + 15% molasses produced the most amount of gas while the treatment with 2.5 g enzyme mixture produced the least amount of gas ($p<0.05$). The high amounts produced gas in treatments with 15% molasses and the treatment with 2.5 g enzyme mixture and 15% molasses is found to be the result of adding molasses which increases the soluble sugar which in turn improves fermentation and all this results in reduction of cell wall and ADF. De Biover et al (2005) have reported that gas production has a reverse relationship with NDF but has a direct relationship with starch which is in agreement with the findings of this research. The results of this study showed that all of the treatments increased fraction b of the reed silage ($P<0.05$). The highest amount of fraction b was seen to be that of the treatment with 2.5 g enzyme mixture + 15% molasses (53.48) and the least amount was that of the control group (48.79). Also, the results of this study showed that using molasses and enzyme mixture in every kg dry matter together would increase fraction b more than when the same amounts of molasses and enzyme mixture are used separately ($P<0.05$). The same trend was found for fraction c and the results showed that all of the treatments have increased fraction c of the reed silage ($P<0.05$). The highest amount of fraction c was that of the treatment with 15% molasses (0.049) and the least amount was that of the control group (0.025). The reason for high amounts of gas production parameters is the result of the increase of molasses fermentability which is in agreement with the findings of Mahala and Khalifa (2007). They reported that increasing molasses in sorghum silage would increase gas production.

TABLE III. PRODUCED GAS (ML IN 200 MG DRY MATTER) AT DIFFERENT HOURS OF INCUBATION AND ITS PARAMETERS

Sig.	SEM	Treatment with 2.5 g enzyme + 15% molasses	Treatment with 15% molasses	Treatment with 2.5 g enzyme	Control group	Incubation time
NS	0.979	5.32	4.21	3.77	3.75	2
NS	1.621	11.10	9.67	8.80	9.05	4
NS	2.13	13.49	11.59	10.57	10.65	6
NS	2.667	16.23	15.14	12.53	12.91	8
NS	3.413	21.28	18.57	16.98	17.33	12
NS	4.27	35.83	32.30	30.50	31.22	24
NS	3.428	45.30	43.60	40.64	42.52	48
NS	3.213	48.80	47.74	44.02	46.93	72
NS	2.723	50.84	50.82	47.28	49.79	96
Gas production properties						
*	1.004	53.48^{a}	49.57^{b}	48.99^{c}	48.79^{c}	b
*	0.0014	0.046^{a}	0.049^{a}	0.039^{b}	0.025^{c}	c
*	0.054	8.10^{a}	7.84^{b}	7.19^{c}	5.87^{d}	ME
*	0.355	61.30^{a}	58.58^{b}	54.40^{c}	46.99^{d}	OMD

*In each row, numbers with dissimilar characters have significant differences.

*Significant difference at the level of (P<0.05).

*NS: not significant; b: slow degradation fraction; c: degradation rate constant; ME: metabolizable energy (MJin kg dry matter); OMD: organic matter degradability (gram in kg dry matter; SEM: standard error of measurement.

D. Organic Matter Degradability (OMD) and Metabolizable Energy (ME)

The amount of digestibility of treatments varied from 61.30 to 46.99. The results of this study showed that processing reed silage with enzyme and molasses would increase the amount of ME and OMD of the experimental groups (P<0.05). The treatment with 2.5 g enzyme + 15% molasses showed the highest amount of OMD (61.30%) and the least amount of OMD was that of the control group (46.99%). The highest amount of ME was that of the treatment with 2.5 g enzyme + 15% molasses and the least amount was that of the control group. Results of the present study showed that using both enzymes and molasses together increased the ME more than when they were used separately. This trend is probably the result of high amounts of sugar in molasses and the degradation of cell wall by fiber degrading enzymes which provides a perfect substrate for ruminal microorganisms to grow in. Arbabi et al (2008) have reported that processing foxtail millet silage with different amounts of molasses increases the ME in comparison with the control group (P<0.05) which is in agreement with the findings of this study.

IV. CONCLUSION

The overall aims of this study were to increase the nutritional value of reed forage silos by adding enzymes and molasses. The results of this study show that using enzymes and molasses could be positively used to process the reed roughage and thus increase its nutritional value. Regarding the chemical components of reed silos' treatments and also considering the OMD and ME, the treatment with 2.5 g enzyme and 15% molasses shows the highest coefficients. On the whole, it is suggested to use the 2.5 g enzyme mixture and 15% molasses treatment to improve the digestibility of this forage and noting the fact that reed is constantly used by Sistani farmers, by adding urea and molasses and then storing them in silos, not only the forage could be used through the whole year, it will also improve its nutritional value and dairy performance.

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